In re Application of:

Montero-Julian, et al.

PATENT
ATTY. DOCKET NO.: BECK1130-2

Application No.: 10/782,664 Filed: February 18, 2004

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## **AMENDMENT**

## Amendments to the Specification:

Following the abstract, please insert the attached Sequence Listing with subsequent page numbering thereafter.

Please replace paragraph [00078] with the following amended paragraph:

as template monomer for the peptide exchange assays. Experiments were carried out with HLA A\*0201 and a series of known peptides. Previous experiments in the laboratory indicated the peptide Mart-1 27-35 (Kawakami, et al., 1994a *Proc. Natl. Acad. Sci. USA*. 91: 3515-3519) as well as peptide Mart-1 26-35 (Kawakami, et al., 1994b *J. Exp. Med.* 180: 347-352), derived from melanoma cells, could be excellent candidates to manufacture monomer template. Both peptides, which have amino acid sequences AAGIGILTV (SEQ ID NO:1) and EAAGIGILTV (SEQ ID NO:2) respectively, have been described as a low and medium affinity peptides for the HLA-A\*0201 molecule (Valmori, et al. 1998 (1998) *J. Immunol.* 161:6956-6962; Kuhns, et al., 1999 *J. Biol. Chem.* 274(51):36422-36427; Men, et al., 1999 *J. Immunol.* 162:3566-3573).

Please replace paragraph [00082] with the following amended paragraph:

[00082] Peptide HBc 18-27 (FLPSDC(FITC)FPSV (SEQ ID NO:3) (Phe-Leu-Pro-Ser-Asp-Cys-Phe-Pro-Ser-Val) (Van der Burg, et al., 1995, 1996) was selected as tracer peptide and different concentrations of the competitor peptide (to be tested for desired peptide specificity). In most of the cases when tetramers were manufactured after the exchange, the competitor peptide was added at 100X fold molar excess. The invention peptide exchange reaction was carried out with the monomers and peptides in solution (10 mM Tris, 150mM NaCl, 0.5 mM EDTA; 0.1% NaN<sub>3</sub>, 0.2% BSA; pH 8.0). The mixture was incubated overnight

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(15 to 20 hours) at 21°C (controlled temperature) under shaking and protected from light. One aliquot was taken to determine the peptide exchange yield. The rest of the sample was tetramerized with the SA-PE as described below.

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Please replace paragraph [00088] with the following amended paragraph:

[00088] HBc 18-27-FITC (FLPSDC(FITC)FPSV) (SEQ ID NO:3) (Van der Burg, et al., 1995 Human Immunol. 44:189-198; and Van der Burg et al. 1996 J. Immunol. 156: 3308), has a tyrosine in position 6 replaced by a cysteine for labelling with the fluorescent dye FITC.

Please replace paragraph [000118] with the following amended paragraph:

[000118] Jurkat P1/1 CD8 clone 5.2 Jurkat P1/1 CD8 clone 5.2 is CD3+, CD4+, CD8+, Vb6.7+. The TCR recognizes the Melan A "wild type" peptide (AAGIGILTV) (SEQ ID NO:1) (Mart-1 27-35), but the decamer (EAAGIGILTV) (SEQ ID NO:2) (Mart-1 26-35) and the mutated peptide (26-35L, also called 27L in the literature (ELAGIGILTV) (SEQ ID NO:4) are better recognized. The same type of cell was prepared without CD8. The CD8 cell (called Jurkat P1/1) recognizes the Melan A "wild type" peptide (AAGIGILTV) (SEQ ID NO:1) with extremely low affinity, and is hardly detectable by flow cytometry. Jurkat 1.1 cells clone 5.2, which is specific for the Mart-1 peptides, 27-35, 26-35 and 26-35L restricted to HLA-A\*0201 were selected because this cell line can be used to quantify the level of functionally of peptide exchange.